Covid-19 and Liver Injury: Role of Inflammatory Endotheliopathy, Platelet Dysfunction, and Thrombosis

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Liver injury, characterized predominantly by elevated aspartate aminotransferase and alanine aminotransferase, is a common feature of coronavirus disease 2019 (COVID-19) symptoms caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Additionally, SARS-CoV-2 infection is associated with acute-on-chronic liver failure in patients with cirrhosis and has a notably elevated mortality in patients with alcohol-related liver disease compared to other etiologies. Direct viral infection of the liver with SARS-CoV-2 remains controversial, and alternative pathophysiologic explanations for its hepatic effects are an area of active investigation. In this review, we discuss the effects of SARS-CoV-2 and the inflammatory environment it creates on endothelial cells and platelets more generally and then with a hepatic focus. In doing this, we present vascular inflammation and thrombosis as a potential mechanism of liver injury and liver-related complications in COVID-19. (*Hepatology Communications* 2022;6:255-269).

Liver injury, primarily consisting of elevated transaminase levels, has been frequently observed in patients with coronavirus disease 2019 (COVID-19) and is correlated with clinical outcomes, including mortality.[1-3] The pathophysiologic mechanism of transaminase elevation, however, remains incompletely defined. The possibility of direct infection of hepatic cells with severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) continues to be a topic of very active research. While controversy exists on this question, overall conclusive evidence of direct viral infection causing liver injury in SARS-CoV-2 is lacking.[4] An alternative mechanism of liver injury in SARS-CoV-2 is endothelial-mediated inflammation and thrombosis. Endothelial inflammation, platelet recruitment, and thrombosis have been implicated in the pathogenesis of nonalcoholic steatohepatitis (NASH)[5,6] and portal hypertension.[7] Here, we present a discussion of endotheliopathy, altered platelet function, and inflammation in COVID-19 and how their synergy may result in liver injury in these patients. We have also summarized clinical (Table 1) and histologic (Table 2) studies related to hepatic coagulopathy in patients with COVID-19.

TABLE 1. LIVER AND COAGULOPATHY IN PATIENTS WITH COVID-19, CLINICAL STUDY

Authors	Patients	Rate of Liver Injury	Patients With Liver Injury Had High Level of				Thrombus	
			ESR	Fibrinogen	D-Dimer	FDP	Portal	Venous
McConnell et al.[94]	3,780	1,006 (27%) (ALT >3×)	NA	X	X	NA	NA	NA
Lu et al.[127]	1,361	125 (9%) (ALT >5× & AST >3×)	NA	NA	X	NA	NA	NA
Wang et al.[128]	657	303 (46%) (ALT >1×)	X	NA	NA	NA	NA	NA
Tsutsumi et al.[129]	60	31 (52%) (ALT >40 U/L)	NA	X	X	X	NA	NA
La Mura et al.[12]	1	1 (ALT 257 U/L)	NA	X	X	NA	X (CT)	
Del Hoyo et al.[130]	1	1 (ALT 1,065 U/L)	NA	NA	X	NA		X (CT)
Franco-Moreno et al.[121]	1	1 (ALT 111 U/L)	NA	X	X	NA	X (CT)	
Abeysekera et al.[131]	1	1 (ALT 55 U/L)	NA	NA	NA	NA	X (CT)	
Borazjani et al.[132]	1	1 (ALT 67 U/L)	NA	NA	NA	NA	X (CT)	
Randhawa et al.[133]	1	None	NA	NA	NA	NA	X (CT)	
Sh Hassan et al.[134]	1	1 (ALT 1,325 U/L)	NA	NA	NA	NA		X (CT)
Kolli and Oza[135]	1	None	NA	NA	NA	NA	X (CT)	
Jeilani et al.[136]	1	1 (ALT 41 U/L)	NA	NA	X	NA	X (CT)	

Abbreviations: AST, aspartate aminotransferase; CT, computed tomography; ESR, erythrocyte sedimentation rate; FDP, fibrin/fibrinogen degradation products; NA, not available; X, present.

Endotheliopathy in COVID-19

While a great deal of focus has centered on COVID-19 as a respiratory disease, it has important systemic manifestations, including on the cardiovascular and immune systems.[8,9] In clinical practice, numerous thrombotic complications have been reported in patients, including deep vein thrombosis, strokes, myocardial infarction, aortic thrombosis, and portal vein thrombosis.[10-13] In addition, the occurrence of a thrombotic event is associated with mortality.[13] Among the features of COVID-19 infection is a coagulopathy characterized by elevated D-dimer and fibrinogen concentrations, with minor changes in prothrombin time and platelet count.[11] Several studies have reported that increased D-dimer is associated with severe COVID-19 and high mortality.[13-16] Furthermore, von Willebrand factor (vWF) activity, vWF antigen, factor VIII activity, and soluble thrombomodulin, a marker of endothelial cell activation, are considerably increased in patients with COVID-19.[11,16] The clinical relevance of these derangements is highlighted by the fact that mortality in patients with COVID-19 is correlated with vWF antigen and soluble thrombomodulin levels.[16] Von Willebrand factor is predominantly produced by endothelial cells, and soluble thrombomodulin is an endothelial transmembrane glycoprotein that is released

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TABLE 2. LIVER AND COAGULOPATHY IN PATIENTS WITH COVID-19, HISTOLOGICAL STUDY

Authors	Patients	Rate of Liver Injury	Thrombus Sinusoidal	Portal	Central Vein
Bugra et al.[137]	98 Postmortem liver tissues	NA		X (2 cases)	
Sonzogni et al.[56]	48 Postmortem liver tissues	25 (61%) (ALT >1×)	X (13 cases)	X (35 cases)	
Kondo et al.[138]	43 Postmortem liver tissues	12 (29%) (ALT >3×)	X (23 cases)		
Lagana et al.[86]	40 Postmortem liver tissues	NA	X (6 cases)		
Fassan et al.[139]	25 Postmortem liver tissues	NA	X (5 cases)	X (3 cases)	
Schurink et al.[140]	21 Postmortem liver tissues	NA	X		
Lax et al.[141]	11 Postmortem liver tissues	4 (36%) (ALT >1×)			X (1 case)
Rapkiewicz et al.[54]	7 Postmortem liver tissues	NA	X (7 cases)		
Fiel et al.[142]	2 Liver biopsy	2 (ALT 2,074 U/L & 2,786 U/L)	X (1 case)		

Abbreviations: NA, not available; X, present.

following endothelial disruption or injury.[17] These findings suggest that endotheliopathy is a critical component of the prothrombotic imbalance in patients with COVID-19 and an important contributory factor to mortality in this disease. To understand the role of endotheliopathy in COVID-19, it is important to understand normal endothelial cell physiology and thrombus formation and how this physiology is altered by the SARS-CoV-2 virus. The role of inflammatory signaling in creating a procoagulant endothelium will be addressed separately.

ENDOTHELIAL CELL FUNCTION AND THROMBUS FORMATION IN GENERAL

In noninflamed tissues, vascular endothelial cells maintain blood fluidity, regulate blood flow, control vessel wall permeability, and maintain quiescence of circulating leukocytes.[18] Thrombosis is the pathological process of blood clot (or thrombus) formation on the inner surface of the endothelium. Blood clots consist of accumulated platelets (platelet plug) and a mesh of cross-linked fibrin.[19] The formation of blood clots involves interplay among various cell types and cascades of coagulation factors.[20] Platelet adhesion to the injured endothelial cells is an early step in thrombosis. Attached platelets are activated by the action of thrombin (also known as activated factor II), which facilitates additional recruitment of circulating platelets to the injury site to form a platelet plug. Thrombin is generated from prothrombin by a series of well-described cascades of coagulation factors. Damaged endothelial cells and hepatocytes produce a procoagulant molecule, tissue factor, that binds and activates circulating procoagulant molecule factor VII. Activated factor VII proteolytically cleaves factor X to form active factor X, which then converts prothrombin to its active form thrombin. In addition to platelet activation and aggregation, thrombin facilitates a cascade of coagulation events to generate fibrin and cross-links fibrin chains to form a large fibrin mesh.[19,21,22]

In normal hemostatic conditions, unnecessary blood clotting is inhibited by multiple antithrombotic factors produced by healthy endothelial cells. First, endothelial cells express tissue factor pathway inhibitor (TFPI), which inhibits the actions of the factor-VIIa–tissue-factor complex. Second, endothelial cells synthesize and display heparan sulfate proteoglycans on their cell surface; these activate antithrombin III to inhibit thrombin generation. Third, endothelial cells also express thrombomodulin, which diverts the activity of thrombin toward activation of protein C, an inhibitor of coagulation. Fourth, endothelial cells suppress platelet activation.[23]

ENDOTHELIAL EFFECTS OF SARS-COV-2 INFECTION

SARS-CoV-2 uses the angiotensin converting enzyme 2 (ACE2) receptor for internalization, aided by transmembrane protease serine 2 protease.[24,25] The ACE2 receptor is expressed in several organs, including

the lung, heart, liver, kidney, and intestine.[26,27] ACE2 receptors are also expressed by endothelial cells.[28] A high level of transcriptomic changes has been reported in both the lymphatic and vascular endothelial cell population in COVID-19 human tissue biopsies from lung, kidney, liver, and heart, using a single-cell and single-nucleus RNA sequencing approach.[29] In this study, however, almost no liver endothelial cells are recognized to express ACE2. Thus, expression of ACE2 in endothelial cells may be tissue specific.

ACE converts angiotensin I to angiotensin II (AngII), while ACE2 converts AngII to angiotensin 1-7 (Ang1-7). AngII binds AngII receptor type 1 (AT1R) and exerts proinflammatory, pro-oxidant, and vasoconstrictive effects. In opposition, Ang1-7 binds to the Mas receptor and mediates anti-inflammatory, antioxidant, and vasodilatory effects.[30-32] The AngII–AT1R axis also activates a disintegrin and metalloprotease 17 (ADAM17). ADAM17 cleaves the membrane-bound interleukin (IL)-6 receptor α (IL-6Rα) (Fig. 1) and membrane-bound tumor necrosis factor alpha (TNFα) on the cell membrane, generating soluble IL-6Rα and TNFα.[32,33] Additionally, AngII induces expression of plasminogen activator inhibitor 1 (PAI-1) in endothelial cells by AT1R. PAI-1 suppresses tissue-type and urokinase-type plasminogen activator function, resulting in suppression of fibrinolysis. The resulting PAI-1/plasminogen activator imbalance creates a hypercoagulable state.[31,34,35] SARS-CoV-2 binding to ACE2 attenuates ACE2 activity through internalization. Attenuation of ACE2 can lead to a dominant AngII effect, including enhanced inflammation and a hypercoagulable state.

OTHER EFFECTS OF COVID-19 ON THE ENDOTHELIUM

SARS-CoV-2 endotheliopathy may be further enhanced by other clinical factors, including hypoxemia (secondary to acute lung injury/acute respiratory distress syndrome [ALI/ARDS]) and hyperfibrinogenemia. Hypoxia attenuates the anticoagulant function of endothelial cells through suppression of thrombomodulin.[36] Further, hypoxia inducible factor (HIF)-1α and HIF-2α may also produce a prothrombotic endothelium. HIF-2α represses TFPI expression.[37,38] Furthermore, expression of PAI-1 is regulated by HIF-1α and HIF-2α, and hypoxia

may up-regulate PAI-1.[37] In addition, hypoxia activates the nuclear factor kappa B signaling pathway in macrophages and neutrophils,[39,40] and HIF-1α up-regulates ADAM17, IL-6, and TNFα.[41,42] Through these multiple pathways, hypoxia may promote further hypercoagulation and inflammation.

During hyperfibrinogenemia, fibrinogen may contribute to endothelial dysfunction.[43] Fibrinogen binds to endothelial cells through the interactions with cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1), integrin α5β1, and integrin α5β3.[44,45] ICAM1 is constitutively present on endothelial cells, but its expression is increased by proinflammatory cytokines. ICAM1 plays an important role in both innate and adaptive immune responses and is involved in the transendothelial migration of leukocytes to sites of inflammation.[46] Hyperfibrinogenemia may promote further inflammation.[47]

Functional assessment of the systemic microvasculature in COVID-19 has produced mixed results. The most commonly used technique for this assessment in patients has been sublingual videomicroscopy. Both microcirculatory impairment[48,49] and intact microcirculatory function[50] have been described by this method in patients with COVID-19. An additional report defined radiographically diagnosed perfusion deficits in the lungs and kidneys of patients with COVID-19, potentially indicative of a systemic microangiopathy.[51] The important role of systemic endotheliopathy beyond the liver in COVID-19 has been reviewed,[52] highlighting the critical interplay between inflammatory factors, endothelial cells, and platelets in disease pathogenesis throughout the body.

Alterations of Platelet Function in COVID-19

Platelet function in COVID-19 is an area of intense interest and very active research. The high rate of thrombotic complications in this disease[53] raised suspicion for a hypercoagulable platelet phenotype, and recent histologic series have confirmed platelet-rich microthrombi in multiple organs, including the lungs, liver, renal, and cardiac microcirculation.[54,55] These studies are concordant with another recently published autopsy series describing thrombosis in the liver sinusoids, suggesting that platelet hyperactivity
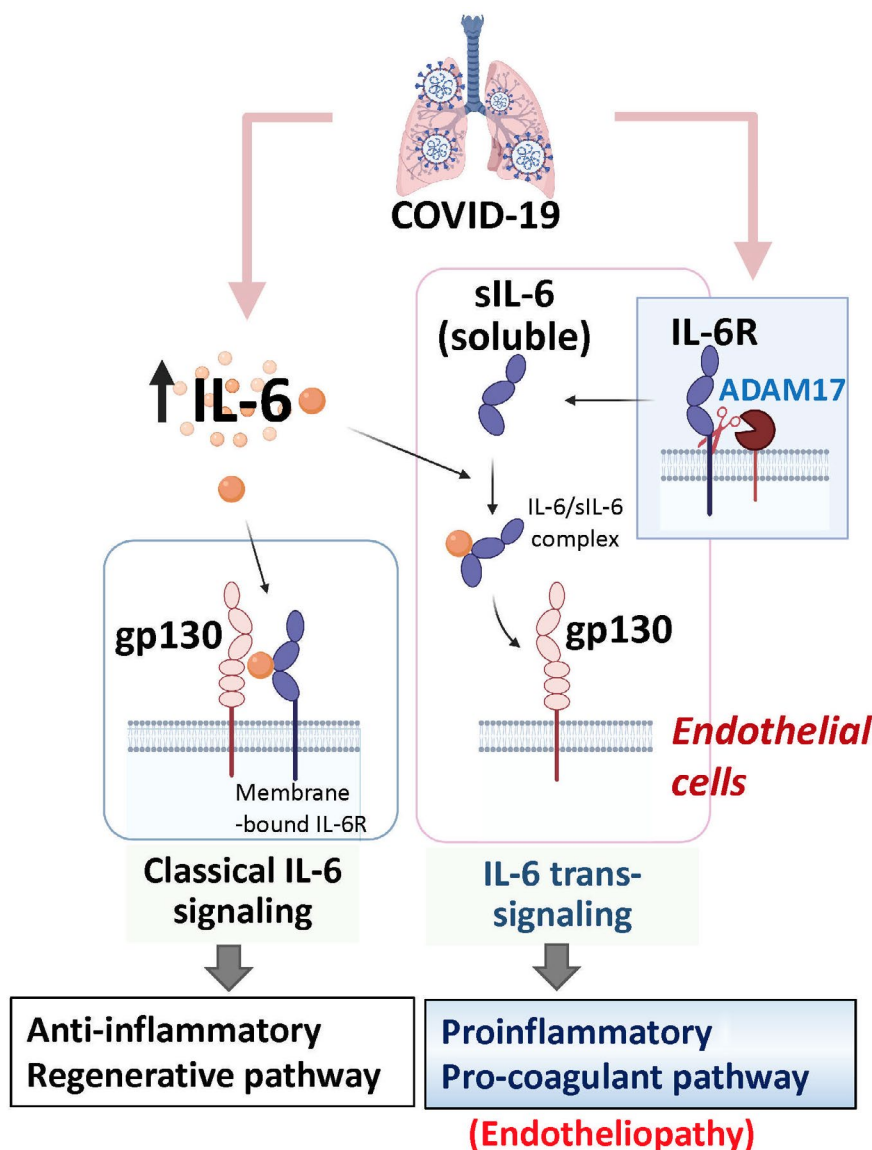
FIG. 1. IL-6 signaling (classical vs. trans-signaling) in COVID-19. Excessive inflammatory cytokine signaling, particularly through IL-6, is thought to be an important factor in the pathogenesis of COVID-19. IL-6 induces downstream signaling through JAK/STAT activation through two pathways. One is classical IL-6 signaling through IL-6 binding to the ligand-binding alpha subunit of its receptor (gp80/IL-6Rα) and subsequently recruiting the signaling beta subunit (gp130) to produce downstream signaling. This pathway is thought to be anti-inflammatory and to promote liver regeneration. The other is trans-signaling that occurs with IL-6 binding to a soluble form of the receptor alpha subunit. ADAM17, which is typically increased in inflammatory conditions, cleaves the membrane-bound IL-6Rα, increasing sIL-6R and creating opportunity for formation of the IL-6/sIL-6R complex, which then interacts with gp130 on target cells that may not express membrane-bound IL-6Ra. IL-6 trans-signaling is thought to be the major route of IL-6 signaling to LSECs and has been implicated in endotheliopathy (proinflammatory and procoagulant state) in COVID-19. The sIL-6R levels are increased in COVID-19,[143] with a likely result of increased trans-signaling. Abbreviation: gp80, interleukin-6 receptor ligand-binding domain.

in COVID-19 may be an important mediator of the frequent liver injury seen in these patients.[56]

The phenotype of platelets in COVID-19 continues to be defined. Recent primate studies of SARS-CoV-2 infection have demonstrated up-regulation of platelet activation in response to infection.[57] Studies in patients have demonstrated increased platelet aggregation on blood smears in severe COVID-19[58] and elevated

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serum markers of platelet activation.[16] Details of platelet function in COVID-19, however, remain controversial. Several reports have demonstrated through RNA sequencing and flow cytometry that platelets from patients with COVID-19 exhibited mitochondrial dysfunction and hyperactivation, as measured by increased P-selectin positivity and aggregation.[59,60] Interestingly, the latter study showed defective generation of "procoagulant" platelets, as measured by annexin V positivity after stimulation, in COVID-19 compared to healthy controls but suggested that platelets unable to form a procoagulant phenotype may hyperaggregate to cause vascular pathology.[60] It has been additionally reported that platelets in COVID-19 demonstrated increased responsiveness to vWF due to increased aggregation in response to ristocetin (a proaggregatory factor[61]) but impaired aggregation in response to agonists, such as adenosine diphosphate, collagen, and arachidonic acid.[62,63]

One area of more broad agreement concerning platelet function in COVID-19 is platelet–leukocyte interactions. In particular, platelet–neutrophil aggregates have been shown to be increased in COVID-19 in several reports[59,64,65] and are likely mediated by up-regulation of platelet P-selectin. Platelet–neutrophil interactions have been shown to mediate neutrophil extracellular trap formation, which is associated with thrombosis, in influenza[66] infection as well as in COVID-19.[65]

Another aspect of platelet phenotype under investigation in COVID-19 is its role in cytokine signaling. In contrast to platelets from healthy donors, platelets from patients with COVID-19 released higher levels of IL-1β, monocyte chemoattractant protein-1/CC motif chemokine ligand 2, and IL-18, among other cytokines, implicating platelets directly in the dysregulated inflammation of the disease.[64]

While platelet dysfunction is becoming increasingly defined as a feature of COVID-19, the specific mechanisms mediating it remain largely unknown, with three main possibilities emerging as candidates: 1) Inflammatory cytokine signaling (e.g., IL-6), 2) toll-like receptor (TLR) signaling, 3) direct infection by platelets with SARS-CoV-2.

INFLAMMATORY CYTOKINE SIGNALING: THE ROLE OF IL-6

Excessive inflammatory cytokine signaling, particularly through IL-6, is thought to be an important factor in the pathogenesis of COVID-19. In addition to its other effects, IL-6 may be mediating COVID-induced coagulopathy by interacting with platelets and contributing to the activated platelet phenotype seen in this disease.[16] In patients receiving IL-6 cytokine therapy for cancer, platelets showed enhanced aggregation and degranulation,[67] and in vitro studies also demonstrated increased platelet activation with IL-6.[68] In addition, plasma IL-6 has been shown to correlate with the abundance of platelet-derived microparticles, which are generated as a consequence of platelet activation.[69] Interestingly, platelets do not possess a membrane-bound form of the IL-6 receptor but do contain the soluble form (sIL-6R), which is released following platelet activation and may provide the manner in which platelets are able to respond to IL-6 through trans-signaling.[70] In a colitis model of experimental inflammation, IL-6 was found to be the key mediator of thrombosis and platelet hyper-reactivity, suggesting a role for IL-6-mediated thrombosis in other disease states as well.[71] The specifics of IL-6 signaling and its effect on platelets in COVID-19 is an area of ongoing research.

TLR SIGNALING

Platelets possess functional TLRs, including TLR7, which recognizes single-stranded RNA and so could provide a possible mechanism for platelet response to SARS-CoV-2. A recent study examining the platelet transcriptomic response to SARS-CoV-2 interestingly found messenger RNA from the virus inside a small number of platelets.[59] This was corroborated in an additional study showing the association of SARS-CoV-2 RNA with platelets.[72] Because platelet expression of ACE2 remains controversial, although it has been described,[73] TLR7 provides a possible alternative mechanism by which viral RNA may enter platelets, and this finding bolsters the suggestion that TLR7-mediated signaling may be playing a role in platelet activation in COVID-19.[59]

DIRECT INFECTION OF PLATELETS

Infection of platelets by SARS-CoV-2 remains an area of controversy and active investigation. Reports
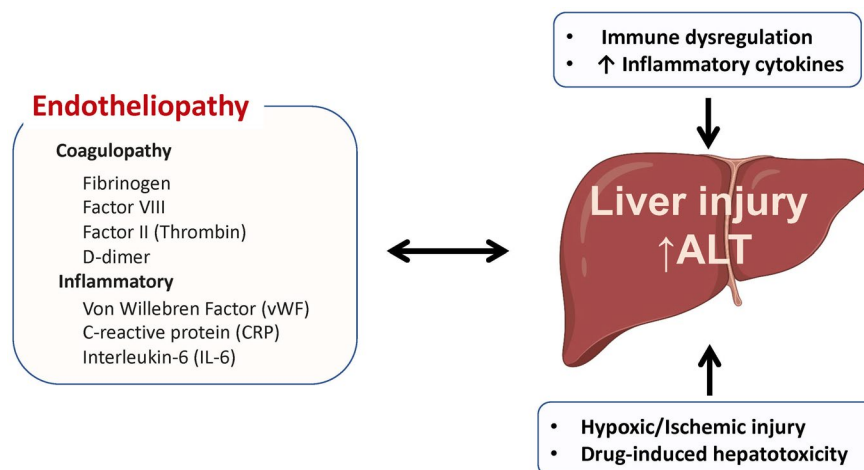
FIG. 2. Potential mechanisms of liver injury in patients with COVID-19. Abbreviation: CRP, C-reactive protein.

have found both that platelets do[73,74] and do not[59] express ACE2, the receptor necessary for SARS-CoV-2 infection. Additional studies are awaited to further define whether direct infection causes platelet abnormalities in COVID-19.

Inflammatory Signaling in COVID-19

Accumulating evidence suggests that the severity of COVID-19 is associated with increased levels of cytokines, such as IL-1β, IL-2, IL-6, IL-8, interferon-γ (IFN-γ)-induced protein 10 (IP-10), TNF-α, IFN-γ, macrophage inflammatory protein 1α and 1β, and vascular endothelial growth factor.[75-77] In previous outbreaks of Middle East respiratory syndrome-coronavirus, a coronavirus related to SARS-CoV-2, proinflammatory cytokines, such as IL-2, IL-6, and IL-8, have been identified[78,79] as key players during infection and possible triggers of liver injury. Del Valle et al.[77] reported that high serum IL-6, IL-8, and TNF-α levels at the time of hospitalization are strong and independent predictors of patient survival in COVID-19 (n = 1,484) and suggested that serum IL-6 and TNF-α levels should be considered in the management and treatment of patients with COVID-19. An additional report identified a triad of IL-6, IP-10, and IL-10 as excellent predictors of a severe disease course in

COVID-19.[80] On the other hand, it was reported that the cytokine profile in patients with severe COVID-19 does not differ from moderate COVID-19, ARDS, and sepsis.[81] However, several important therapeutic differences between COVID-19-associated cytokine storms and many other cytokine storm disorders have been reported.[82] Notably, although blood clotting disorders can occur throughout a cytokine storm, thromboembolic events seem to occur more frequently in cytokine storms associated with COVID-19.[83]

Liver Injury and COVID-19

LIVER INJURY AS A MANIFESTATION OF ENDOTHELIOPATHY, PLATELET DYSFUNCTION, AND INFLAMMATION IN COVID-19

The mechanism of liver injury in COVID-19 infection is likely multifactorial and associated with immune dysregulation and cytokine storm, hypoxic/ischemic injury, and drug-induced hepatotoxicity.[84,85] Understanding the hepatic manifestations of the coagulopathy of COVID-19 will give additional insight into liver injury in this disease (Fig. 2). One recent series of postmortem liver biopsies from patients with COVID-19 reported portal or sinusoidal vascular thrombosis in at least 50% of patients.[56] High D-dimer values were also almost

universally present in the cohort of patients (96%), and elevated alanine aminotransferase (ALT) was also highly prevalent (62%), suggesting a relationship between liver vascular thrombosis, coagulopathy, and liver injury.[56] Additional pathology of COVID-19 livers at autopsy has also demonstrated platelet-fibrin microthrombi in the hepatic sinusoids along with some portal vein platelet aggregates. In some cases, these findings were associated with ischemic-type damage in the liver.[54] Another series of liver pathology at autopsy demonstrated what were termed "thrombotic bodies." These were inclusions in the sinusoids that were positive for a platelet marker, clusters of differentiation 61 (CD61).[86]

Interestingly, a common feature of liver pathology in COVID-19 seems to be hepatic steatosis. As demonstrated in models of nonalcoholic fatty liver disease (NAFLD), activation of coagulation is capable of driving hepatic steatosis, and this may be a novel mechanism linking the thrombosis and steatosis that are prevalent in the livers of patients with COVID-19.[87]

The role of PAI-1 in COVID-19 liver injury is potentially interesting as elevated PAI-1 has been associated with NAFLD and NASH.[88,89] PAI-1 is known as a marker of endothelial cell injury,[90] and overproduced PAI-1 binds to TLR4 on macrophages, inducing the secretion of proinflammatory cytokines and chemokines.[91] Recently, PAI-1 has been demonstrated to be elevated due to IL-6 signaling to vascular cells in COVID-19,[92] and this occurrence in the liver may contribute to microvascular thrombosis, inflammation, and steatosis.

IL-6 SIGNALING (CLASSICAL VS. TRANS-SIGNALING) AND ENDOTHELIOPATHY

Beyond its effect on PAI-1, IL-6 has been reported to be a key factor in acute liver injury in COVID-19.[79] IL-6 signals to cells either directly through the membrane-bound IL-6 ligand-binding domain (gp80) and the signal-transducing component of the receptor (gp130) on the same cell or by trans-signaling, in which a soluble form of the ligand-binding receptor domain binds IL-6 and complexes with gp130 alone on the cell membrane[92,93] (Fig. 1). Liver sinusoidal endothelial cells are not thought to express the membrane-bound ligand-binding domain for IL-6. We reported that IL-6 trans-signaling induced liver sinusoidal endotheliopat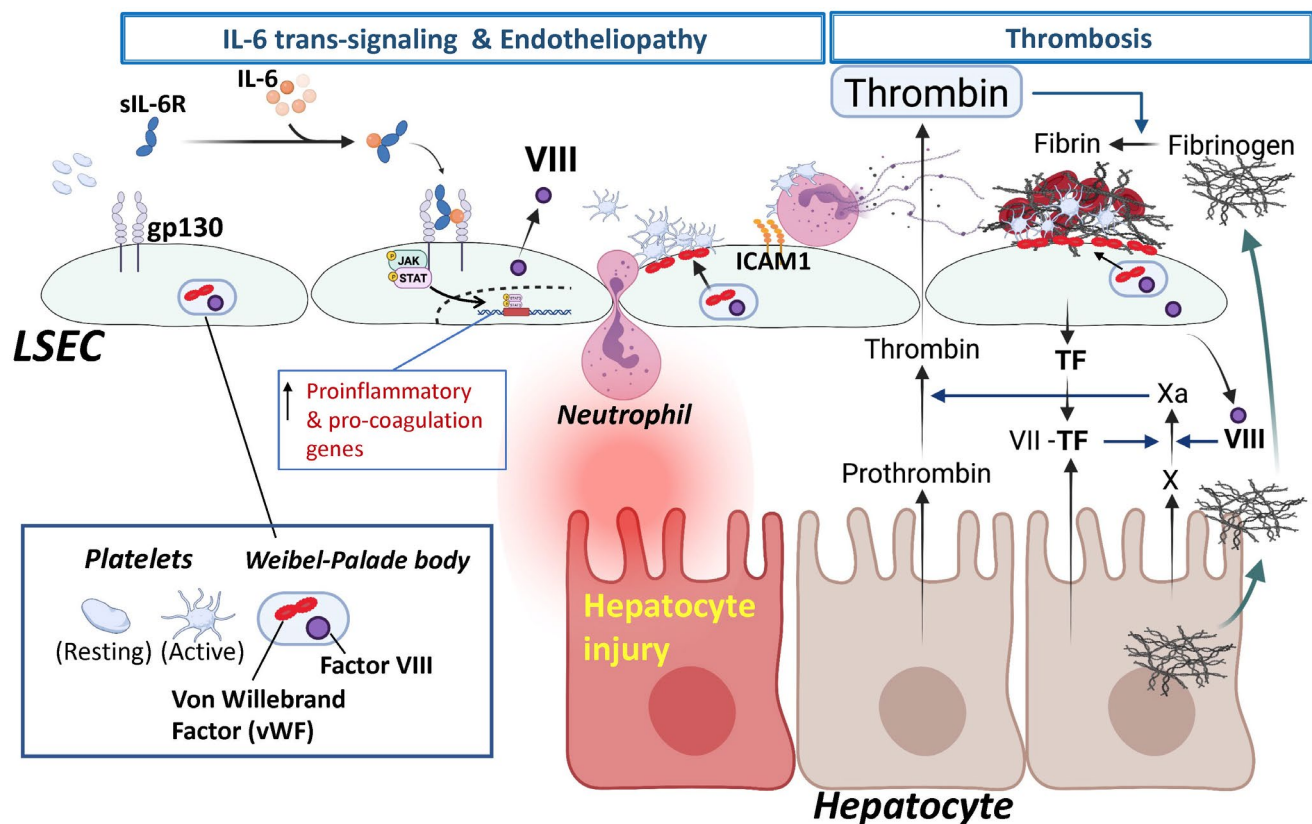hy with neutrophil infiltration and a hypercoagulable liver sinusoidal endothelial cell (LSEC) phenotype[94] (Fig. 3). Patients with COVID-19 with liver injury demonstrated elevated levels of IL-6, serum markers of hypercoagulability, increased LSEC dysfunction on histology as evidenced by increased vWF, and increased platelet accumulation and neutrophil infiltration in the liver. Because of the association of liver injury with both elevated IL-6 and a hypercoagulable and inflammatory LSEC phenotype, further experiments were done exploring the role of IL-6 trans-signaling in LSECs. These studies demonstrated that IL-6 trans-signaling produces a hypercoagulable and inflammatory LSEC phenotype through Janus kinase-signal transducer and activator of transcription (JAK/STAT) activation, blockable with JAK inhibitors. This suggests that LSEC endotheliopathy may be an important mechanism by which liver injury occurs in COVID-19. More broadly, elevated plasma ICAM1, indicative of endothelial damage, has been recently defined as a predictor of mortality in patients with both cirrhosis and COVID-19 or sepsis.[95] IL-6 trans-signaling induced ICAM1 up-regulation by LSECs in our study, indicating that this pathway of inflammatory endotheliopathy may be broadly important for key outcomes in our patients with infection and inflammation.

IL-6 may also provide an interesting link between poor outcomes observed in alcohol-related liver disease and COVID-19. IL-6 has been shown to be up-regulated by ethanol consumption and in alcohol-related liver disease.[96,97] While IL-6 is a potent factor for inducing liver regeneration[98] and may in some cases play a protective role in the liver,[99] it has also been associated with poor outcomes in acute-on-chronic liver failure (ACLF),[100,101] suggesting a complex physiology. Given the proinflammatory effects of IL-6 trans-signaling in the liver vasculature and the frequent observation of hepatic vascular pathology in COVID-19, up-regulated inflammatory IL-6 signaling may be contributing to poor outcomes in alcohol-related liver disease.

TYPE I IFNS

Interesting results have also been obtained in liver organoids exposed to inflammatory cytokines typical of COVID-19. Type I IFNs have been shown in this setting to up-regulate ACE2 expression on hepatocytes and facilitate SARS-CoV-2 infection. Further studies are awaited to define this process *in vivo* in patients infected with SARS-CoV-2.[102]

FIG. 3. Liver sinusoidal endotheliopathy and liver injury in COVID-19. IL-6 trans-signaling and endotheliopathy: IL-6 is highly elevated in patients with COVID-19 and is related to liver injury. LSECs are not thought to express the membrane-bound ligand-binding domain for IL-6. However, the complex of IL-6 and sIL-6R binds to gp130 on the cell membrane, inducing IL-6/JAK/STAT signaling, known as IL-6 trans-signaling. The IL-6 trans-signaling induces liver sinusoidal endotheliopathy with neutrophil infiltration and a hypercoagulable LSEC phenotype. IL-6 trans-signaling increases expression of proinflammatory (ICAM1, CXCL1, CXCL2, P-selectin, and E-selectin) and procoagulation genes (factor VIII and vWF). Weibel-Palade body includes vWF and factor VIII in LSECs. IL-6 trans-signaling promotes movement of vWF to the LSEC surface, facilitating platelet attachment, an initial step of the thrombus formation. Endotheliopathy is associated with neutrophil recruitment, potentially leading to hepatocyte injury. Platelet–neutrophil interactions mediate NET formation. Microvascular thrombosis: Thrombus (blood clots) consists of accumulated platelets (platelet plug) and a mesh of cross-linked fibrin. Thrombosis involves interplay among various cell types and cascades of coagulation factors. Platelet adhesion to the injured LSECs is an early step in thrombosis. Attached platelets are activated by the action of thrombin (also known as activated factor II), which facilitates additional recruitment of circulating platelets to the injury site to form a platelet plug. Thrombin is generated from prothrombin by a series of well-described cascades of coagulation factors. Damaged endothelial cells and hepatocytes produce a procoagulant molecule, TF, which binds and activates circulating procoagulant molecule factor VII. Activated factor VII proteolytically cleaves factor X to form Xa, which then converts prothrombin to its active form, thrombin. In addition to platelet activation and aggregation, thrombin facilitates a cascade of coagulation events to generate fibrin and cross-links fibrin chains to form a large fibrin mesh. Patients with COVID-19 show an elevated fibrinogen level. Abbreviations: CXCL, chemokine (C-X-C motif) ligand 1; NET, neutrophil extracellular trap; TF, tissue factor; Xa, active factor X.

BILIARY INJURY AND CHOLESTASIS IN COVID-19

In addition to a hepatocellular pattern of enzyme elevation, liver injury in COVID-19 can manifest with a cholestatic pattern as well.[103,104] This has been hypothesized to be due to ACE2 expression on biliary epithelial cells leading to direct viral infection. Interestingly, however, two reports of cholestasis following COVID-19 infection suggest a possible role for endotheliopathy, noting hepatic artery branches in the portal tract with endothelial swelling with luminal narrowing, portal vein endophlebitis (inflammation of the intima area of a vein), and endothelialitis (leukocyte

attachment to the vascular wall) and thrombotic material in portal vein branches.[103,105] COVID-19 cholestasis has been reported as a long-term complication of infection,[105] leading to the need for liver transplantation[106] and highlighting its importance for further mechanistic study.

COVID-19 THERAPEUTICS AND LIVER INJURY

Current therapeutics for COVID-19 that may impact endotheliopathy and liver injury include those targeting the virus itself (remdesivir), IL-6 and its downstream signaling pathways (tocilizumab and baricitinib), and systemic inflammation (dexamethasone). One report suggests that dexamethasone may ameliorate endothelial injury in COVID-19.[107] The nuclear receptor subfamily 3 group C member 1 (NR3C1) receptor, a key target of dexamethasone, has also been shown by single-cell RNA sequencing to be coexpressed with IL-6 in endothelial cells, suggesting dampening of endothelial IL-6 production as a key action of corticosteroids in COVID-19.[108] Administration of the IL-6 receptor antagonist tocilizumab has been suggested to improve coagulation parameters in COVID-19, which may indicate improved endothelial function as well.[109] The combination of remdesivir with the JAK inhibitor baricitinib was recently shown to improve outcomes in COVID-19 compared to remdesivir alone,[110] and baricitinib was also shown to improve COVID-19 outcomes compared with standard of care, including dexamethasone and remdesivir.[111] Treatment with a JAK inhibitor would be expected to ameliorate IL-6-mediated endotheliopathy in the liver. A major limitation in applying these therapeutic approaches to liver injury, however, is the frequent exclusion of patients with aspartate aminotransferase or ALT above 5 times the upper limit of normal from the studies and the association of remdesivir, tocilizumab, and baricitinib with liver injury.[112-114] We speculate that application of therapy to prevent endothelial-mediated liver injury would need to occur early in the course of hospitalization, but this should be clarified in future studies. Given the lack of data showing a benefit of directing therapy toward liver injury in COVID-19 and the risks of the therapies themselves to the liver and the patient overall, more data are needed to determine which, if any, patients with liver injury require specific treatment.

Conclusion: Consequences of Liver Injury in COVID-19

COVID-19 disease in patients with preexisting liver disease has been a topic of active research, and vascular pathology in the liver may be particularly important to the outcomes of these patients. In patients with cirrhosis, SARS-CoV-2 infection has been associated with both decompensation and ACLF.[115,116] While the specific mechanisms of decompensation remain under investigation, vascular dysfunction due to the endotheliopathy and platelet activation described above could be playing a role in worsening portal hypertension in these patients.

In addition to the acute setting, the long-term consequences of liver injury in COVID-19 remain unknown. The question of long-term effects has been given additional importance by evidence that COVID-19 viral antigens may be present in liver tissue up to 6 months following recovery.[117] In the lungs, fibrosis seems to be a consequence of COVID-19 disease,[118] and basic models of pulmonary fibrosis have implicated activation of coagulation in this process.[119] Whether this occurs in the liver remains unknown. Multiorgan proteomics recently completed in patients with COVID suggests that profibrotic pathways are up-regulated in the liver due to COVID-19, and follow-up data are needed for evaluation of any long-term consequences.[120] In one case, portal vein thrombosis occurred in a patient without cirrhosis after resolution of SARS-CoV-2 infection with negative real-time polymerase chain reaction,[121] suggesting ongoing endotheliopathy and coagulopathy. A study of survivors of acute SARS-CoV-2 infection found that patients had an elevated risk of liver test abnormalities when followed for months after their infection, suggesting some possible long-term sequelae for the liver.[122] Additional reports have raised the possibility of persistent liver inflammation and liver fat accumulation as detected by magnetic resonance imaging following COVID-19[123] and the potential for increasing liver stiffness over time following COVID-19,[124] which must be confirmed in future studies. Metabolomic profiling of patients recovered from COVID-19 also demonstrates elevated taurine concentrations, which

are potentially indicative of liver injury, 3 months after recovery, and further study in this area will be of interest.[125] The mechanism of any long-term liver injury remains speculative, but endotheliopathy has been reported to be sustained following COVID-19,[126] suggesting endothelial-mediated inflammation as a possible mechanism. Long-term consequences of liver injury in COVID-19 will require ongoing study and observation of patients.

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